

Millicomposting Versus Vermicomposting: A Statistical Comparison Of The Quality Of The Resulting Organic Composts

Lorena Gonzaga Dobre Batista

Universidade Federal de Sao Paulo

Leda Lorenzo Montero (✉ leda.lorenzo@unifesp.br)

Universidade Federal de Sao Paulo <https://orcid.org/0000-0002-5853-6751>

Mirian Chieko Shinzato

Universidade Federal de Sao Paulo <https://orcid.org/0000-0002-9238-339X>

Research Article

Keywords: Organic fertilizer, Diplopoda, Trigonius corallinus, Earthworms, Eisenia foetida

Posted Date: October 21st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-924696/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The efficiency of millicomposting (M, with millipedes), vermicomposting (V – with earthworms), and traditional composting (C – no invertebrates) of vegetal waste was investigated statistically. Composting took place in closed systems to avoid external interferences during the experiments and allow monitoring of the main physico-chemical parameters. The experiments were replicated six times ($n = 6$). Quality was assessed via analysis of variance (one-way and Welch procedures) and post-hoc comparisons. Temperature profiles were similar in the three composting types. After 92 days, the compost volume produced in V (51%) decreased more than in M (43%) and C (44%) ($p = 0.001$). Organic carbon, nitrogen contents, and C/N ratios were also similar (all $p > 0.1$). Vermicomposting produced humus of higher nutritional quality, whereas M leachates yielded higher nutritional levels and maturity degrees. The Ca content was higher in V and M, while K and Mg were higher in V. pH, Ca, Mg, P_2O_5 , and S contents were higher in V than in C (all $p < 0.05$). The leachate volumes, electrical conductivity, and Na^+ and PO_4^{3-} contents were similar in the three composting types ($p > 0.05$). pH, K^+ and NH_4^+ contents were higher while the NO_3^- was lower in V than in M (all $p < 0.005$). The only difference observed in C was lower pH when compared to those of V. Although all three composting types were efficient in producing mature, high-quality organic fertilizers, the addition of detritivorous animals improved the composting efficiency and the quality of the final products. This study also attested the potential of millicomposting in producing good-quality liquid fertilizers.

Statement Of Novelty

The quality of milli- and vermicomposting was assessed by statistical analysis. Statistical analysis confirms the importance of replication in studies related to composting. The addition of invertebrates in composting increases Ca^{2+} contents in humus and leachates. The nutrient contents in the vermicompost humus were higher, while the millicompost leachate yielded higher nutrient levels and maturity degree (NH_4^+/NO_3^-).

1. Introduction

About 1.3 billion tons or 1/3 of all the food produced for human consumption is lost or wasted per year around the world [1]. Latin America and the Caribbean region are in the fourth position in the rank of biggest food waste countries in the world, with 11.6%, whereas the world's average is 13.8% [2]. In Brazil, the organic fraction represents more than 50% of waste generation [3] and its recycling is still incipient, especially in households [4]. The final disposal of solid waste in this country is mostly in sanitary landfills (59.5%), followed by controlled landfills (23.0%), and dumpsites (17.5%) [5]. Furthermore, inadequate disposal favors the proliferation of disease vectors and the contamination of soil, water and air, because the generation of leachate and the emission of methane gas [6]. This inefficient management totally invalidates the potential value of organic waste to become an alternative fertilizer by composting [5].

Composting is a biological process of controlled aerobic decomposition that transforms organic waste into nutrient-rich and stable organic products [7], which can be used as agricultural fertilizers [8–11]. This technique is especially interesting in countries, such as Brazil, where agriculture is one of the pillars of the economy, in addition to being highly dependent on the use of synthetic fertilizers.

To optimize composting, temperature, humidity and oxygenation must be properly controlled. Increasing temperatures stimulate the activity of decomposers. This is achieved by controlling the volume of the composting piles – the higher the volume, the higher the temperature [12]. However, it is not always possible to maximize volume in medium and small-scale composting. It is the case of households, which are the main source of food waste generation [13–14]. The use of closed systems is most appropriate for household waste composting because of the relatively small size, continuous waste production and limited space [12].

In addition, detritivorous fauna can increase the efficiency of composting, especially in small spaces and/or closed systems. Invertebrates specialized in detritus fragmentation may triturate waste, promoting faster bacterial activity. They add mucilaginous substances that can ameliorate the physicochemical quality of the resulting products [15]. The use of such invertebrates is especially interesting in (a) systems with no contact with soil and where animals do not naturally colonize; (b) composting of very fibrous materials, such as sugarcane bagasse; and (c) small to medium-scale composting, carried out in small-volume piles or digesters, such as in household waste composting.

Earthworms are probably the most studied and used soil organisms in composting (vermicomposting), along with microbial inoculants. Earthworms excrete products that constitute nutritious organic fertilizers, which are rich in humus, stabilized organic matter, macro and micronutrients, beneficial soil microorganisms, and growth hormones [7, 15, 16]. These substances have proved to be promoters and protectors of various plant crops in pot experiments [8–11, 15]. Furthermore, according to [17], the application of composts (from composted domestic waste) can increase the amount of humidified carbon in agricultural soils for four years.

Millipedes also seem to perform an interesting role in composting, since they are specialized in the consumption of vegetal detritus, being the most active saprophages in decomposition [18–22]. They act in pedogenesis and nutrient cycling, producing organic substances that enrich the soil with macro and micronutrients [18, 19]. Such capacity is probably due to its intestinal microbiota, which breaks down cellulose into simple sugars [20]. This symbiosis gives to millipedes the potential to compost wastes with high cellulose content, such as sugarcane bagasse. The positive influence of millicomposts on plant growth and fruit production has also been reported [19, 21, 23, 24], indicating the potential of millicomposting to produce agricultural fertilizers of good quality. At this point, it is important to note that millipedes do not transmit any type of disease, unlike other invertebrates of the class Diplopoda [25].

Studies conducted with earthworms [9–11, 15, 16], and millipedes [19, 22–24] reported that composts produced by invertebrates are of good quality and stimulate plant growth. However, millicomposting is still underrated. As far as we know, there are no reports on the quality of leachates produced in

millicomposting, nor on its agricultural use. In addition, there are scarce comparisons between vermi- and millicomposting, which makes it difficult to optimize invertebrate-mediated composting and to standardize guidelines. To our knowledge, there are only two published studies comparing the efficiency of both invertebrates simultaneously under controlled conditions. One of them had no replication at all [18], and the other had only two samples of each composting type ($n = 2$) [20]. Therefore, it is not possible to identify significant differences, nor to extrapolate results from these studies. Thus, there is the need of a comparative evaluation to elucidate the differences between earthworm- and millipede-mediated composting. Furthermore, it is mandatory to perform controlled experiments with robust designs, to analyze the data via statistical comparisons, with obvious gains in inference power and evaluation of the significance of the results. In addition, it is necessary to expand our knowledge about millicomposting, generating data on the quality of its products, particularly leachates.

Considering the state-of-the-art, the present study aims to assess the use of invertebrates in composting using a robust experimental design. The efficiency of composting is assessed by the analysis of the maturation and the nutritional quality of the resulting compounds (solids and liquids) in three closed-system scenarios: vermicomposting (V), millicomposting (M), and traditional composting (C), with no addition of detritivorous animals. Replication was conducted six times ($n = 6$), in order to apply statistical methods of data analysis and validation.

2. Materials And Methods

2.1. Preparation of materials and monitoring systems

Closed systems were used in the experiments in order to control the main parameters that can affect the organic matter decomposition, such as aeration and humidity, and to simulate small- and medium-scale composting. Three composting systems were prepared: vermicomposting (V) – containing earthworms; millicomposting (M) – containing millipedes, and traditional composting (C) – with no addition of detritivorous animals (invertebrates). It was also used as a control system. Each closed system was prepared using two 18-liter plastic bins, one placed on top of the other. Holes of 3 mm in diameter were drilled at the bottom and top of the upper bin (digester box), which was then covered. The upper part of the lower bin (leachate collector box) was opened and in contact with the upper bin.

Six closed systems (replicates) were prepared for each composting type (V, M and C), resulting in 18 composting systems ($n = 6$). All the 18 closed systems were maintained under the same environmental conditions and the same type and proportion of organic waste was stored in each of them. They were systematically displayed in a row in an aerated place, protected from rain and exposed to the same temperature, incidence of sunlight, and wind conditions (Fig. 1). A liter of soil was added to all 18 digester boxes. In the experiments with V and M, about 0.5 L of *Eisenia foetida* earthworms (Oligochaeta: Lumbricidae) and *Trigoniulus corallinus* millipedes (Diplopoda: Trigoniulidae) were respectively added (Fig. 2). Subsequently, manually chopped and homogenized, 2-cm sized waste of low (wood sawdust and sugarcane bagasse) and high (mix of vegetables and fruit leftovers, such as lettuce, cabbage, cauliflower,

papaya, banana, pineapple etc.) C/N ratios was added as 500 mL intercalated layers (in proportions of 1:1) until 4/5 of the upper bin volume was filled.

Figure 1. Bins prepared for the vermicomposting (V), millicomposting (M) and traditional composting (C) experiments replicated six times

Figure 2. Earthworms (a) and millipedes (b) used in vermicomposting and millicomposting, respectively

To guarantee aerobic conditions, the waste layers were revolved weekly in the first month and the volumes recorded. Ambient temperature and the temperatures inside the digester boxes were measured before the waste was revolved. Temperatures were monitored daily in the first month and then, two to three times a week using a glass thermometer, until completing three months (92 days). Finally, the waste volume was calculated by measuring the height of the waste pile with a tape placed inside the digester boxes.

2.2. Physico-chemical characterization of composting products

After 92 days the final composting products were prepared for chemical characterization. Solid compounds (humus) were dried at room temperature and sieved (2 mm aperture), while the leachates were filtered.

Humus samples were analyzed for pH (via the potentiometric method using $0.01 \text{ mol L}^{-1} \text{ CaCl}_2$ solution), density (mass/volume), moisture (from mass loss at $60\text{--}65^\circ\text{C}$), organic carbon (OC, by dichromate oxidation followed by titration), total nitrogen (N, by sulfuric digestion using the Kjeldahi method), phosphorus (P_2O_5 , via spectrophotometry using a vanadomolybdate solution), potassium (K_2O , via flame photometry using a vanadomolybdate solution), sulfur (S, by barium sulfate gravimetric method), calcium (Ca) and magnesium (Mg) (by extraction with HCl followed by atomic absorption spectrophotometry). These analyses were carried out at *Laboratório de Análise de Fertilizantes e Corretivos da Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo*. To estimate the maturity of the composting products, C/N ratios were calculated. The methods used follow the Manual of Official Methods for Mineral, Organic and Corrective Fertilizers by MAPA [26]. To assess the quality of the composting products obtained in this study, our results were compared to the parameters required by Normative Instruction SDA/MAPA # 61/2020 [27] for solid organic fertilizers.

Leachate samples produced in the three composting systems were analyzed for final volume, pH (pH meter HI 2221/Hanna Instruments), electrical conductivity (EC) (EC meter HI 2250/Hanna Instruments), sodium (Na^+) and potassium (K^+) (flame photometer DM-63 /Digimed), ammonium (NH_4^+) (Nessler method and UV-Vis spectrophotometer Genesys 20/Thermo Scientific), nitrate (NO_3^-) and phosphorus (P_2O_5^+) contents (photometer HI 83215/Hanna Instruments). The maturity degree of the leachates was

estimated using $\text{NH}_4^+/\text{NO}_3^-$ ratios [28]: $\text{NH}_4^+/\text{NO}_3^-$ ratios < 0.5 indicate very high maturity; ratios between 0.5 and 3 indicate maturity, and > 3 , low maturity [7].

2.3. Statistical analysis

Differences between composting V, M and C were assessed via analysis of variance, using one-way ANOVA when the assumptions were satisfied. Homogeneity of variances and normal distributions were assessed via Levene and Shapiro-Wilk tests, respectively. For variables with heterogeneous variances (NO_3^- contents and pH of leachates only), the differences were detected using the Welch test, as it is robust in these cases, as long as the samples are balanced [29]. In case of significant differences ($p < 0.05$), post-hoc tests were used to determine which treatments differed, using the Tuckey test for variables with normal distribution and the Dunn test with Bonferroni correction for non-normal ones (NO_3^- contents and pH of leachates). Statistical analysis was performed using the *Past Version 3.25* software.

3. Results

3.1. Temperature and volume evolution during composting

Temperatures were similar over 92 days for the three composting types (Fig. 3). The values were close to ambient temperature, which remained between 12 and 26 °C throughout the experiment, performed during the winter (Fig. 3). Temperatures inside the digester boxes were above the ambient temperature only during the first 18 days. The highest temperature (28.8°C) was reached on the 12th day, and it was only 2 °C above the ambient temperature (Fig. 1). Diplopods seemed to be more sensitive to low temperatures than earthworms, as their activity decreased during the coldest periods (temperatures $\leq 10^\circ\text{C}$).

Figure 3. Temperature evolution for the three composting types (means and standard deviations; $n = 6$). The dashed line marks the ambient temperature

After the 92nd day, the final volume loss (Fig. 4) was greater in V than in M and C ($p = 0.000673$). The volume loss was fast until the 25th day and stabilized between the 32nd and 67th days. There was a volume increase between the 67th and 74th days. Then, the volume loss continued until the 92nd day, when the experiment was finished. The average loss at the end of the experiment was 51.4% (± 2.30) in V, 42.9% (± 3.51) in M and 43.8% (± 2.98) in C (Fig. 4).

Figure 4. Volumes of the three composting types during 92 days (means and standard deviations; $n = 6$)

3.2. Chemical quality of the solid compounds (humus)

The moisture content of the solid products of the three composting types ranged from 42 to 49% and pH was neutral. All of them contained relevant amounts of nutrients and reached maturity with C/N ratios below 20 (Table 1).

Table 1
Chemical quality of the solid compounds (humus) obtained from the three composting types and the values required by the national legislation for the sale of solid organic fertilizers [20].

Parameters	Control	Vermicompost	Millicompost	Legislation
OC (%)	25.55 ± 2.2	27.74 ± 2.8	28.47 ± 6.2	≥ 15
N (%)	1.67 ± 0.43	1.75 ± 0.33	1.89 ± 0.18	≥ 0.5
P ₂ O ₅ (%)	0.37 ± 0.04	0.48 ± 0.06	0.43 ± 0.04	*
K ₂ O (%)	1.41 ± 0.24	1.67 ± 0.25	1.23 ± 0.18	
Ca (%)	1.71 ± 0.16	2.25 ± 0.18	2.35 ± 0.21	≥ 1.0**
Mg (%)	0.16 ± 0.01	0.21 ± 0.02	0.18 ± 0.01	≥ 1.0**
S (%)	0.18 ± 0.07	0.29 ± 0.03	0.24 ± 0.05	≥ 1.0**
Moisture (%)	44.57 ± 8.76	48.96 ± 8.49	42.44 ± 13.50	≤ 50
pH	7.08 ± 0.47	7.72 ± 0.12	7.42 ± 0.25	*
C/N	16 ± 3.10	16 ± 3.33	15 ± 4.17	≤ 20

Mean and standard deviation values for n = 6. Humidity at 65°C. *As stated in the registration record by the manufacturer or importer. ** Minimum levels of secondary macronutrients, when present in the product.

The P₂O₅, K₂O, Ca, Mg, and S contents and pH differed among the composting types (p-values reported in Fig. 5). The other parameters (OC, N, C/N ratio, density and humidity at 65°C) did not differ significantly (all p-values > 0.1).

Figure 5. Chemical parameters obtained for the solid organic compounds (humus) resulting from the three composting types (V-vermicomposting, M-millicomposting and C-control). Boxplots show median and quartile values (n = 6). ANOVA p-values indicated for each variable. Different letters indicate post-hoc significant differences (p < 0.05) according to the Tuckey test (Dunn test for pH)

Calcium contents were higher in M and V when compared to C. The other chemical parameters were higher in V when compared to C (except for K₂O, with similar contents in V and C). Vermicomposting resulted in higher K₂O and Mg contents when compared to those resulting from millicomposting (Fig. 5). All solid compounds obtained from M, V and C are within the Brazilian legislation requirements for solid organic fertilizers [27].

3.3. Chemical quality of the liquid compounds (leachates)

Similar final volumes, electrical conductivity, and Na^+ and P_2O_5^+ contents were obtained for the leachates resulting from the three composting types (p-values were 0.232; 0.213; 0.858 and 0.068 respectively). However, there were quality differences (p-values reported in Fig. 6). Higher NO_3^- contents were observed in M leachates, while pH, K^+ and NH_4^+ contents were higher in V. The only difference observed between M and V (addition of invertebrates) and C was the lower pH values in V (Fig. 6).

Figure 6. Physicochemical parameters of leachates resulting from the three composting types (V-vermicomposting, M-millicomposting and C-control). Boxplots show median and quartile values (n = 6). ANOVA *p*-values indicated for each variable. Different letters indicate post-hoc significant differences ($p < 0.05$) according to the Tuckey test (corrected Dunn test for pH and NO_3^-)

Nutrient contents were high in the leachates (Table 2). $\text{NH}_4^+/\text{NO}_3^-$ ratios of 0.93 were obtained for V, 0.008 for M, and 0.08 for C, indicating that the V leachate is mature (0.5-3) and M and C leachates are highly mature (< 0.5) [7]. V leachates (and humus) were more alkaline than M and C leachates.

Table 2
Chemical quality of leachates obtained from three composting types.

Parameter	Control	Vermicomposting	Millicomposting
Volume (mL)	436 ± 201.72	507 ± 161.51	342 ± 101.08
EC (d/S m ⁻¹)	6.08 ± 0.73	6.65 ± 0.34	5.73 ± 1.25
Ph	8.64 ± 0.17	9.20 ± 0.08	8.19 ± 0.38
Na ⁺ (mg L ⁻¹)	84.28 ± 16.72	89.78 ± 17.15	86.22 ± 18.13
K ⁺ (mg L ⁻¹)	1648.72 ± 347.49	2315.06 ± 492.03	1291.89 ± 243.32
NO ₃ ⁻ (mg L ⁻¹)	266.67 ± 152.02	33.33 ± 55.78	944.44 ± 547.18
NH ₄ ⁺ (mg L ⁻¹)	21.53 ± 9.45	31.05 ± 11.35	8.29 ± 3.38
P ₂ O ₅ ⁺ (mg L ⁻¹)	122.20 ± 40.37	150 ± 54.77	188.89 ± 40.37
NH ₄ ⁺ /NO ₃ ⁻	0.08	0.93	0.01
Mean and standard deviation values for n = 6.			

4. Discussion

4.1. Temperature and volume evolution during composting

Temperature patterns along the 92-day period were similar for the three composting types and very close to the ambient temperature. The observed temperatures were relatively low (12 to 26 °C), probably because the experiment was carried out in winter. The low waste volume disposed in the digesters (about 14 L) can also account for these results, since surface/volume ratio of composting waste is inversely related to composting temperature [12]. It is worthy to remark that the volumes tested in the present study are suitable for medium- to small-scale composting systems.

The maximum temperature observed was 28.8 °C, which is about 20 °C below the expected for a common thermophilic phase, in which high temperatures contribute to eliminate pathogens. However, the invertebrates are temperature sensitive, and the temperature range from 20 to 28 °C must be appropriate for vermicomposting [30]. On the other hand, vermicomposting has been shown to reduce pathogens when compared to traditional high-temperature composting, which appears to be related to earthworm digestion [31]. Therefore, the temperatures obtained in our study were appropriate to the survival of the invertebrates and, at the same time, to produce humus and leachates of high quality and maturity.

Volume loss was higher in vermicomposting (51%) than millicomposting (43%), indicating higher efficiency of earthworms in reducing organic waste. Similarly, previous works had related volume losses of 60% and 40% for organic waste treated by vermicomposting and millicomposting, respectively [20, 32]. These results reinforce the potential use of this type of composting in households, which waste a large part of the food produced for consumption [13, 14].

Volume profiles in the three systems were also very similar. At the beginning, volume loss was rapid, and the temperature inside the digesters was higher than the ambient temperature thanks to the activity of microorganisms in decomposing easily degradable organic matter. After volume stabilization in approximately 40 days, there was a brief volume increase, maybe related to the manual revolving of the waste to promote aeration. From the 72nd day on, volume continued to slowly decrease, reflecting the intensification of humification and final maturation of the composts. The performance of the invertebrates was more evident at the end of the 92-day period, resulting in the highest volume loss in vermicomposting, highest leachate maturity in millicomposting, and gains in chemical quality, which will be discussed below.

4.2. Nutritional content of solid compounds (humus)

The nutritional content was higher in V, when compared to those of M and C. However, the three composting types produced nutrient-rich organic compounds of neutral pH and C/N ratios < 20, indicating their potential as fertilizers. In fact, these compounds comply with the Brazilian legislation for organic fertilizers [4].

Calcium contents were higher in V and M, when compared to C. This Ca content increase may be explained by the secretion of calcium carbonate granules produced by earthworm calciferous glands around their esophagus during vermicomposting [33, 34]. In millicomposting, the incorporation of

calcified parts of the exoskeletons, such as millipede cephalic capsules, may respond for Ca increase in M products [35]. This addition of exoskeleton parts (exuviae) may occur during ecdysis and after death.

Excretion of calcium-rich feces by microorganisms in the intestines of the invertebrates can also contribute to the Ca increase in the humus [36]. Calcium is probably in the form of carbonates and/or oxides. Earthworm secretions, for example, contain calcium carbonate, calcite, aragonite, vaterite and amorphous calcium carbonates [33, 34]. These Ca-rich substances are suitable for agricultural use, especially when applied to acid soils, such as the Oxisols, which are common in tropical areas and predominate in Brazil.

Mg and K₂O contents were higher in V than in M, whereas P₂O₅ and S were higher in V than in C. Nitrogen contents were similar in the three composting types. Such results differ from those reported by Thakur et al. [18], who found higher K₂O and P₂O₅ contents in the milli- than vermicomposting, and higher N and P₂O₅ contents in milli- and vermicomposting than in traditional composting [18]. These apparent contradictions seem to be related to the type of materials used in composting, since these authors used a mixture containing animal manure (soil, cow dung, vegetable skins, leaves, grass and rice straw), which are rich in N, probably resulting in low C/N ratios. Regarding the invertebrates, they used different species, such as the diplopoda *Harpaphe Haydeniana* for millicomposting and the earthworm *Eudrilus eugeniae* for vermicomposting. In addition, this previous study did not replicate the composting experiments, and the conclusions were drawn from a single set of experiments. Therefore, it is not possible to check whether the differences reported by them are significant.

4.3. Potential effects of the types of waste in composting with invertebrates

There are some evidences of the influence of the type of waste in the efficiency of composting with invertebrates [37] compared millicomposts (prepared using millipedes *Arthrosphaera magna*) to vermicomposts reported in the literature. They observed that final products of the Areca waste vermicomposting yielded higher pH values and a lower organic carbon content; however, these differences did not occur when composting coconut waste.

Differences in composting mediated by earthworms and millipedes may be related to their food preferences, which in turn, depend on the use of resources from which they evolved. Earthworms are adaptable to a wide range of environments, since there are more than 3,500 species described. Epigeic earthworms, as *Eisenia foetida*, are “soil-formers” living at the interface between the forest floor and the soil surface; they can consume decomposing organic matter (vegetal debris and animal feces) [38]. Therefore, they are able to consume waste with high N concentration, such as animal manure. In symbiosis with their intestinal microorganisms, they produce and excrete coprolites, which are stabilized-organic-matter rich feces (high humic substances content) [15, 35].

Diplopodes, on the other hand, are litter transformers living on the forest floor they are saprophages specialized in the consumption of vegetal debris, such as leaf, grass and wood litter [23, 39] of high C/N

ratios and structural carbon contents in the form of cellulose and lignin. During digestion, millipedes crush, moisten and inoculate the material with microorganisms. In this case, microbial activity in the feces after excretion is important to the complete detritus degradation [35].

Both types of invertebrate act as catalysts during composting and respond to the quality of the substrate. For example, there is a greater biomass of both earthworms and millipedes in litter and soil in forest plantations of low C/N ratios than in plantations of high C/N ratios [40]. However, millipedes are almost restricted to the consumption of litter layers, while the earthworms access a wide variety of resources both on the litter and on the soil surfaces. Thus, millipedes may be more sensitive than earthworms to the palatability of the decomposing materials, which would partly explain our results.

Indeed, literature indicates that millicomposting should be performed only with vegetal waste, simulating what happens in forest soils [20, 21, 32, 37]. Since millipedes' food preferences are plant waste, their use can be optimized in urban composting of pruning waste, which has similar characteristics to litter. Despite the material used in the present study was of vegetal origin, half of it was composed of fresh vegetable waste from local fruit and vegetable markets. This type of material (water- and N-rich, of low C/N ratios) may have favored the activity of earthworms to the detriment of millipedes. On the other hand, the material of high C/N ratios used in this study was rich in cellulose, hemicellulose, and lignin (mixture of sugarcane bagasse and sawdust), being more difficult to degrade. Apparently, this material was unattractive to the invertebrates, explaining the leftovers found in the digester boxes at the end of the experiments.

Further studies should evaluate the potential use of earthworms and millipedes in degrading urban solid waste of different chemical characteristics (C/N ratios, proportions of cellulose, lignin, fibers and polyphenols), to understand their waste-dependent efficiency. Vermicomposting is considered an efficient method to produce high quality composts from different types of animal manure [7, 15, 16]. It could contribute to the degradation of waste containing feces of domestic animals. On the other hand, the composting of pruning waste can be optimized by reducing the particle size and/or adding waste of high energy content, such as food waste [41]. Therefore, the use of millipedes plus food waste could be a good technical solution for the composting of pruning waste.

4.4. Degree of maturity and quality of vermi- and millicomposting

Stability and maturity of composting products evolve together, although they are not quite the same thing. Stability is related to the activity of decomposing microorganisms, while maturity refers to the potential of the composting product to promote plant growth, which, in turn, depends on the stabilization of organic matter. Maturity can be evaluated by pH (from > 7.0 to ≤ 8.0) and C/N ratios (from $< 10/1$ to $> 20/1$) [7,36]. The pH of matured composts tends to be neutral to alkaline, reaching values higher than 8.0 due to the formation of humic acids that react with basic chemical elements forming alkaline humates [42]. The C/N ratio considered as indicator of maturation vary: the Brazilian legislation adopts C/N ratios

≤ 20 for organic composts [27]. Despite the lack of consensus on the ideal minimum value, this parameter reflects the capacity of microorganisms to degrade organic material.

In composting, stability begins after the initially fast mass loss. At the beginning of the stability phase, the transformation of materials that are difficult to degrade, such as lignin, still occurs, giving rise to the complexification of organic matter. Thus, small mass loss oscillations may occur in the stability phase, during the maturation of the composts. In this process, humic acids increase, fulvic acids decrease and the C/N ratio increases [7]. This improves the quality of the compost. Therefore, long maturation phases tend to generate higher quality composts, with more stable organic matter. It is not possible to indicate in this study when exactly composts reached maturity, because the humus quality was only assessed at the end of the experiments. However, we can affirm that three months was time enough to achieve maturation and to produce high-quality composts, appropriate for use as fertilizers. The humus obtained in this study complies with the maturity degree parameters pH (7.1–7.7) and C/N ratio (15–16) which, according to Bernal et al. [7], they must be around 6.6 and 7.8 and 10.8 to 19.3, respectively.

4.5 Degree of maturity and chemical quality of liquid compounds (leachates)

The species of invertebrates used in composting affected the quality of leachates. The M leachates yielded higher N (in the form of NO_3^-) contents, higher maturation indexes (lower $\text{NH}_4^+/\text{NO}_3^-$ ratios), and lower alkalinity. The V leachates yielded higher K^+ contents and a very alkaline pH value (9.2). Besides, V leachates yielded very low N contents and almost half in the form of NH_4^+ , which increased the $\text{NH}_4^+/\text{NO}_3^-$ ratios of V leachates relatively to those of M and C leachates. Furthermore, four of the six V leachate samples did not yield any NO_3^- , probably because in such alkaline conditions, nitrogen was completely lost [7]. These differences may reflect the influence of the metabolism of each invertebrate, related to processes occurring in their digestive tracts, as discussed in Sect. 4.3.

The V products (humus and leachates) were the most alkaline. The pH values obtained for the three types of leachates were moderately alkaline, but higher than those reported in the literature, which varied between 6 and 7.9 [8, 9, 43]. This can be related with the type of composting material, since in those previous studies waste of animal origin was used. Humus and leachate pH and C/N ratios are usually higher in composts derived from vegetal waste [8, 9, 16]. Although pH increases indicate maturity, very high pH can be harmful for vegetal growth. Liquid fertilizers have optimal pH values varying from 5.5 to 8, because for $\text{pH} > 7.5$, nitrogen losses can occur due to NH_4^+ volatilization [7]. Consequently, high pH values can explain the low N levels obtained for their leachates. Considering that NO_3^- is the most available N form for vegetation, our results point out that M leachates are more adequate as liquid fertilizers, since they yielded higher NO_3^- contents and lower (almost neutral) pH. Nonetheless, all the leachates obtained in this study are for use as agricultural fertilizers. Provided that it is diluted, V leachates can be used as fertilizer. Indeed, there are reports of their positive effects on germination, plant growth, and fruit quality [9–11, 43]. On the other hand, high concentrations can have inhibitory effects

because of excessive alkalinity and salt concentrations, and dilutions above 10% are not recommended [10].

Potassium concentrations were low in solid composts and high in leachates, thanks to its extreme mobility. The K contents are 2 to 4 times higher in leachates than those reported in the literature, while phosphorus contents were lower [8, 9, 44]. This probably reflects the high K content of the sugarcane bagasse used in this study [45], and the fact of using waste exclusively of vegetal origin. Previous studies support this explanation, since they report differences in chemical quality according to the type of waste used in composting, suggesting that initial C/N ratios are decisive [8, 9, 46].

5. Conclusions

Millicomposting, vermicomposting and traditional composting (with no addition of invertebrates) were compared, in order to assess their efficiency and the quality of their products (humus and leachates). Vegetal waste was degraded in replicated ($n = 6$), controlled experiments, carried out in small (18L) and closed digesters, emulating household-scale composting systems for 92 days. For each composting type, the variability between digesters was relatively high, which remarks the importance of replication in composting studies.

Temperature profiles were similar for the three composting types and followed the ambient temperature variations, probably because the small volume of digesters. Temperatures were appropriate to avoid mortality of invertebrates and to achieve complete waste degradation. Final volumes after 92 days were lower in vermicomposting, indicating higher efficiency in the presence of earthworms. Their use increased the nutritional quality of the humus, while the use of millipedes increased that of the leachates, which yielded higher degree of maturity ($\text{NH}_4^+/\text{NO}_3^-$ ratios).

All humus and leachates reached maturation. The use of invertebrates increased calcium contents in the composting products. Invertebrate-mediated composting produced high quality, nutrient-rich, neutral to moderately alkaline products of C/N ratios below 20, indicating potential use as agricultural fertilizers, complying with the Brazilian legislation.

Finally, the relatively high variations of experimental results point to the importance of replicating the composting experiments. To compare one or two experimental results per composting type is not enough to accurately assess the efficiency of the process; replication is mandatory to evaluate the significance of possible differences between treatments.

For future studies, it is recommendable to investigate invertebrate-mediated composting according to the type of waste, comparing vermicomposting and millicomposting efficiencies using waste of contrasting chemical characteristics.

Declarations

ACKNOWLEDGMENTS

The authors thank the Núcleo de Apoio Técnico ao Ensino, Pesquisa e Extensão of Instituto de Ciências Ambientais, Químicas e Farmacêuticas da Universidade Federal de São Paulo (NATEPE-ICAQF/UNIFESP) for supporting some experimental work.

Funding - This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest/Competing interests (include appropriate disclosures) - Not applicable.

Availability of data and material (data transparency) - Not applicable.

Code availability (software application or custom code) - Not applicable.

Authors' contributions: Lorena Gonzaga Dobre Batista: Investigation, Writing - Original Draft; Leda Lorenzo Montero: Conceptualization; Experimental design; Writing - Original Draft; Supervision; Mirian Chieko Shinzato: Conceptualization; Writing - Original Draft; Supervision; Project administration.

References

1. Food and Agriculture Organization of the United Nations (FAO): Global food losses and food waste – Extent, causes and prevention. Rome, Italy. 37 p. (2011)
2. Food and Agriculture Organization of the United Nations (FAO): The State of Food and Agriculture 2019. Moving forward on food loss and waste reduction. Rome. Licence: CC BY-NC-SA 3.0 IGO: (2019)
3. Ministério do Meio Ambiente (MMA): Compostagem Doméstica, Comunitária e Institucional de Resíduos Orgânicos: (2017). http://www.mma.gov.br/images/arquivo/80058/CompostagemManualOrientacao_MMA_2017-06-20.pdf. Accessed 2 Feb 2018
4. Brasil. Ministério do Meio Ambiente (MMA): Plano Nacional de Resíduos Sólidos. Versão Preliminar. Brasília: Ministério do Meio Ambiente: (2020). <http://consultaspublicas.mma.gov.br/planares/wp-content/uploads/2020/07/Plano-Nacional-de-Res%C3%ADduos-S%C3%B3lidos-Consulta-P%C3%BAblica.pdf>. Accessed 13 Sept 2020
5. Associação Brasileira de Empresas de Limpeza Pública e Resíduos Especiais (ABRELPE): Panorama dos Resíduos Sólidos no Brasil 2018/2019: (2019). <http://abrelpe.org.br/download-panorama-2018-2019/>. Accessed 24 Feb 2020
6. Ministério do Meio Ambiente (MMA): Gestão de Resíduos Orgânicos: (2018). <http://www.mma.gov.br/cidades-sustentaveis/residuos-solidos/gest%C3%A3o-de-res%C3%ADduos-org%C3%A2nicos>. Accessed 19 Feb 2018

7. Bernal, M.P., Alburquerque, J.A., Moral, R.: Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* **100**(22), 5444–5453 (2009).
<https://doi.org/10.1016/j.biortech.2008.11.027>
8. Tejada, M., Gonzales, J.L., Hernandez, M.T., Garcia, C.: Agricultural use of leachates obtained from two different vermicomposting processes. *Bioresour. Technol.* **99**(14), 6228–6232 (2008).
<https://doi.org/10.1016/j.biortech.2007.12.031>
9. Singh, R., Gupta, R.K., Patil, R.T., Sharma, R.R., Asrey, R., Kumar, A., Jangra, K.K.: Sequential foliar application of vermicompost leachates improves marketable fruit yield and quality of strawberry (*Fragaria × ananassa* Duch.). *Sci. Hortic.* **124**(1), 34–39 (2010).
<https://doi.org/10.1016/j.scienta.2009.12.002>
10. Gutiérrez-Miceli, F.A., Llaven, M.A.O., Nazar, P.M., Sesma, B.R., Álvarez-Sólis, J.D., Dendooven, L.: Optimization of vermicompost and worm-bed leachate for the organic cultivation of radish. *J. Plant Nutr.* **34**(11), 1642–1653 (2011). <http://dx.doi.org/10.1080/01904167.2011.592561>
11. Akinnuoye-Adelabua, D.B., Steenhuisen, S., Bredenhanda, E.: Improving pea quality with vermicompost tea and aqueous biochar: Prospects for sustainable farming in Southern Africa. *S. Afr. J. Bot.* **123**, 278–285 (2019). <https://doi.org/10.1016/j.sajb.2019.03.009>
12. Abdullah, N., Chin, N.L., Mokhtar, M.N., Taip, F.S.: Effects of bulking agents, load size or starter cultures in kitchen-waste composting. *Int. J. Recycl. Org. Waste Agric.* **2**, 10 p. (2013).
<https://link.springer.com/article/10.1186/2251-7715-2-3>
13. Tonini, D., Albizzati, P.F., Astrup, T.F.: Environmental impacts of food waste: Learnings and challenges from a case study on UK. *Waste Manage.* **76**, 744–766 (2018).
<https://doi.org/10.1016/j.wasman.2018.03.032>
14. United Nations Environment Programme (UNEP): Food Waste Index Report 2021. Nairobi. 100p. (2021)
15. Adhikary, S.: Vermicompost, the story of organic gold: A review. *Agric. Sci.* **3**(7), 905–917 (2012).
<https://doi.org/10.4236/as.2012.37110>
16. Bhat, S.A., Singh, J., Vig, A.P.: Earthworms as organic waste managers and biofertilizer producers. *Waste Biomass Valor.* **9**, 1073–1086 (2018). <https://doi.org/10.1007/s12649-017-9899-8>
17. Adani, F., Genevini, P., Ricca, G., Tambone, F., Montoneri, E.: Modification of soil humic matter after 4 years of compost application. *Waste Manage.* **27**(2), 319–324 (2007).
<https://doi.org/10.1016/j.wasman.2006.04.004>
18. Thakur, P.C., Apurva, P., Sinha, S.K.: Comparative study of characteristics of biocompost produced by millipedes and earthworms. *Adv. Appl. Sci.* **2**(3), 94–98: (2011).
<http://www.imedpub.com/articles/comparative-study-of-characteristics-of-biocompost-produced-by-millipedes-and-earthworms.pdf> Accessed 21 Apr 2019
19. Karthigeyan, M., Alagesan, P.: Millipede composting: a novel method for organic waste recycling. *Recent Res. Sci. Technol.* **3**(9), 62–67: (2011).
<http://updatepublishing.com/journal/index.php/rrst/article/view/784>. Accessed 21 Apr 2019

20. Ramanathan, B., Alagesan, P.: Evaluation of millicompost versus vermicompost. *Curr. Sci.* **103**(2), 140–143: (2012). <https://www.currentscience.ac.in/Volumes/103/02/0140.pdf>. Accessed 2 Nov 2020
21. Ambarish, C.N., Sridhar, K.R.: Production and quality of pill-millipede manure: a microcosm study. *Agric. Res.* **2**(3), 258–264 (2013). <https://doi.org/10.1007/s40003-013-0075-5>
22. Sridhar, K.R., Ambarish, C.N.: Pill millipede compost: A viable alternative to utilize urban organic solid waste. *Curr. Sci.* **104**, 1543–1547: (2013). <https://www.currentscience.ac.in/Volumes/104/11/1543.pdf> Accessed 2 Nov 2020
23. Antunes, L.F.S., Vaz, A.F.S., Silva, M.S.R.A., Correia, M.E.F., Cruvinel, F.F., Martelleto, L.A.P.: Millicompost: sustainable substrate for the production of dragon fruit seedlings (*Selenicereus undatus*). *Cleaner Eng. Technol.* **4**, 100–107 (2021). <https://doi.org/10.1016/j.clet.2021.100107>
24. Antunes, L.F.S., Souza, R.G., Vaz, A.F.S., Ferreira, T.S., Correia, M.E.F.: Evaluation of millicomposts from different vegetable residues and production systems in the lettuce seedling development. *Org. Agr* (2021). <https://doi.org/10.1007/s13165-020-00342-y>
25. Kania, G., Klapiec, T.: Seasonal activity of millipedes (Diplopoda) – their economic and medical significance. *Ann. Agric. Environ. Med.* **19**(4), 646–650: (2012). https://pdfs.semanticscholar.org/13e4/ef7_ea397081aac9dad939818d2de15943ba.pdf. Accessed 21 Apr 2019
26. Brasil. Ministério da Agricultura, Pesca e Pecuária (MAPA): Manual de Métodos Analíticos Oficiais para Fertilizante e Corretivos. MAPA Secretaria de Defesa Agropecuária. Brasília: MAPA: (2017). http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumosagricolas/fertilizantes/legislacao/manual-de-metodos_2017_isbn-978-85-7991-109-5.pdf. Accessed 21 Apr 2019
27. Brasil: Instrução Normativa nº 61, de 8 de julho de 2020. Ministério da Agricultura Pecuária e Abastecimento. Estabelece as regras sobre definições, exigências, especificações, garantias, tolerâncias, registro, embalagem e rotulagem dos fertilizantes orgânicos e dos biofertilizantes, destinados à agricultura, Brasília, DF, 8 jul. 2020. Ed. 134. Seção 1, p. 5 (2020). <https://in.gov.br/web/dou/-/instrucao-normativa-n-61-de-8-de-julho-de-2020-266802148>. Accessed 12 Sept 2020
28. Sánchez-Monedero, M.A., Roig, A., Paredes, C., Bernal, M.P.: Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. *Bioresour. Technol.* **78**(3), 301–308 (2001). [https://doi.org/10.1016/S0960-8524\(01\)00031-1](https://doi.org/10.1016/S0960-8524(01)00031-1)
29. Liu, H.: Comparing Welch's ANOVA, a Kruskal-Wallis test and traditional ANOVA in case of Heterogeneity of Variance. Thesis of Master of Science Degrees, Faculty of Virginia Commonwealth University, Biostatistics PG Program, 46 p., Richmond, Virginia: (2015)
30. Lourenço, N.M.G.: Características da minhoca Epígea *Eisenia foetida* – benefícios, características e mais-valias ambientais decorrentes de sua utilização. 5 p. Lisboa, Portugal: (2010)

31. Soobhany, N., Mohee, R., Garg, V.K.: Inactivation of bacterial pathogenic load in compost against vermicompost of organic solid waste aiming to achieve sanitation goals: A review. *Waste Manage.* **64**, 51–62 (2017). <https://doi.org/10.1016/j.wasman.2017.03.003>
32. Antunes, L.F.S., Scoriza, R.N., Silva, D.G., Correia, M.E.F.: Production and efficiency of organic compost generated by millipede activity. *Cien. Rural.* **46**(5), 815–819 (2016). <https://doi.org/10.1590/0103-8478cr20150714>
33. Gago-Duport, L., Briones, M.J.I., Rodríguez, J.B., Covelo, B.: Amorphous calcium carbonate biomineralization in the earthworm's calciferous gland: Pathways to the formation of crystalline phases. *J. Struct. Biol.* **162**(3), 422–435 (2008). <https://doi.org/10.1016/j.jsb.2008.02.007>
34. Lee, M.R., Hodson, M.E., Langworthy, G.: Earthworms produce granules of intricately zoned calcite. *Geology.* **36**(12), 943–946 (2008). <https://doi.org/10.1130/G25106A.1>
35. Correia, M.E.F., Oliveira, L.C.M.: Importância da fauna de solo para a ciclagem de nutrientes. In: Aquino, A.M. de; Assis, R.L. de (Ed.). *Processos biológicos no sistema solo-planta: ferramentas para uma agricultura sustentável*. Brasília, DF: Embrapa Informação Tecnológica; Seropédica: Embrapa Agrobiologia, cap. 4. p. 77–99 (2005)
36. Kiehl, E.J.: *Fertilizantes orgânicos*. Agronômica Ceres. 492 p. Piracicaba, São Paulo (1985)
37. Ashwini, K.M., Sridhar, K.R.: Breakdown of plantation residues by pill millipedes (*Arthrosphaera magna*) and assessment of compost quality. *Curr. Sci.* **90**(7), 954–959: (2006). https://www.currentscience.ac.in/Downloads/article_id_090_07_0954_0959_0.pdf. Accessed 2 Nov 2020
38. Dominguez, J., Gómez-Brandón, M.: Ciclos de vida de las lombrices de tierra aptas para el vermicompostaje. *Acta Zoológica Mexicana (n.s.)*. (2), 309–320: (2010). <https://core.ac.uk/download/pdf/234126925.pdf>. Accessed 22 Oct 2020
39. Coleman, D.C., Crossley, D.A., Hendrix, P.F.: Secondary Production: Activities of Heterotrophic Organisms - The Soil Fauna. *Fundamentals of Soil Ecology*. 79–185: (2004). <https://doi.org/10.1016/B978-012179726-3/50005-8>
40. Warren, M.W., Zou, X.: Soil macrofauna and litter nutrients in three tropical tree plantations on a disturbed site in Puerto Rico. *Forest Ecol. Manag.* **170**(1–3), 161–171 (2002). [https://doi.org/10.1016/S0378-1127\(01\)00770-8](https://doi.org/10.1016/S0378-1127(01)00770-8)
41. Reyes-Torres, M., Oviedo-Ocanã, E.R., Dominguez, I., Komilis, D. Sánchez, A.: A systematic review on the composting of green waste: Feedstock quality and optimization strategies. *Waste Manage.* **77**, 486–499 (2018). <https://doi.org/10.1016/j.wasman.2018.04.037>
42. Kiehl, E.J.: *Manual de Compostagem: maturação e qualidade do composto*. Embrapa Meio Ambiente (CNPMA), p. 171. p. Piracicaba, São Paulo (1998)
43. Gutiérrez-Miceli, F.A., García-Gómez, R.C., Oliva-Llaven, M.A., Montes-Molina, J.A., Dendooven, L.: Vermicomposting leachate as Liquid fertilizer for the cultivation of sugarcane (*Saccharum* Sp), J. *Plant Nutr.* (2017). <http://dx.doi.org/10.1080/01904167.2016.1193610>

44. Gutiérrez-Miceli, F.A., García-Gomez, R.C., Rincón, R.R., Abud-Archila, M., Llaven, M.A.O., Cruz, M.J.G., Dendooven, L.: Formulation of a liquid fertilizer for sorghum (*Sorghum bicolor* (L.) Moench) using vermicompost leachate. *Bioresour. Technol.* **99**(14), 6174–6180 (2008).
<https://doi.org/10.1016/j.biortech.2007.12.043>
45. Medina, N.H., Branco, M.L.T., Silveira, M.A.G., Santos, R.B.B.: Dynamic distribution of potassium in sugarcane. *J. Environ. Radioac.* **126**, 172–175 (2013).
<http://dx.doi.org/10.1016/j.jenvrad.2013.08.004>
46. Martins, D.S., Shinzato, M.C., de Moraes, J.E.F.: Evaluation of the use of organic waste generated at hydroelectric power plants in the production of organic fertilizers. *Waste Biomass Valor.* **11**, 5041–5051 (2020). <https://doi.org/10.1007/s12649-019-00790-y>

Figures



Figure 1

Bins prepared for the vermicomposting (V), millicomposting (M) and traditional composting (C) experiments replicated six times

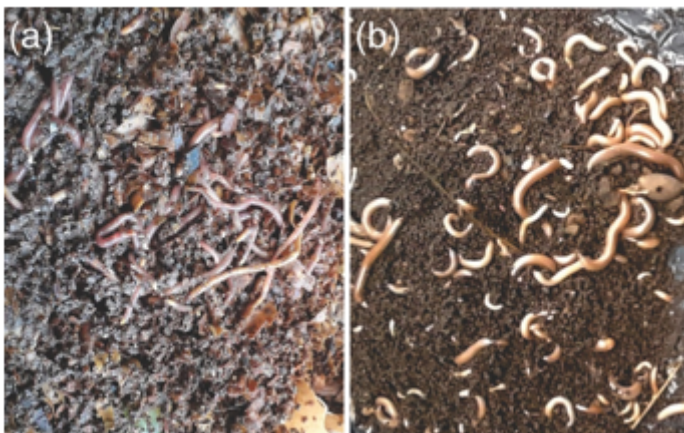


Figure 2

Earthworms (a) and millipedes (b) used in vermicomposting and millicomposting, respectively

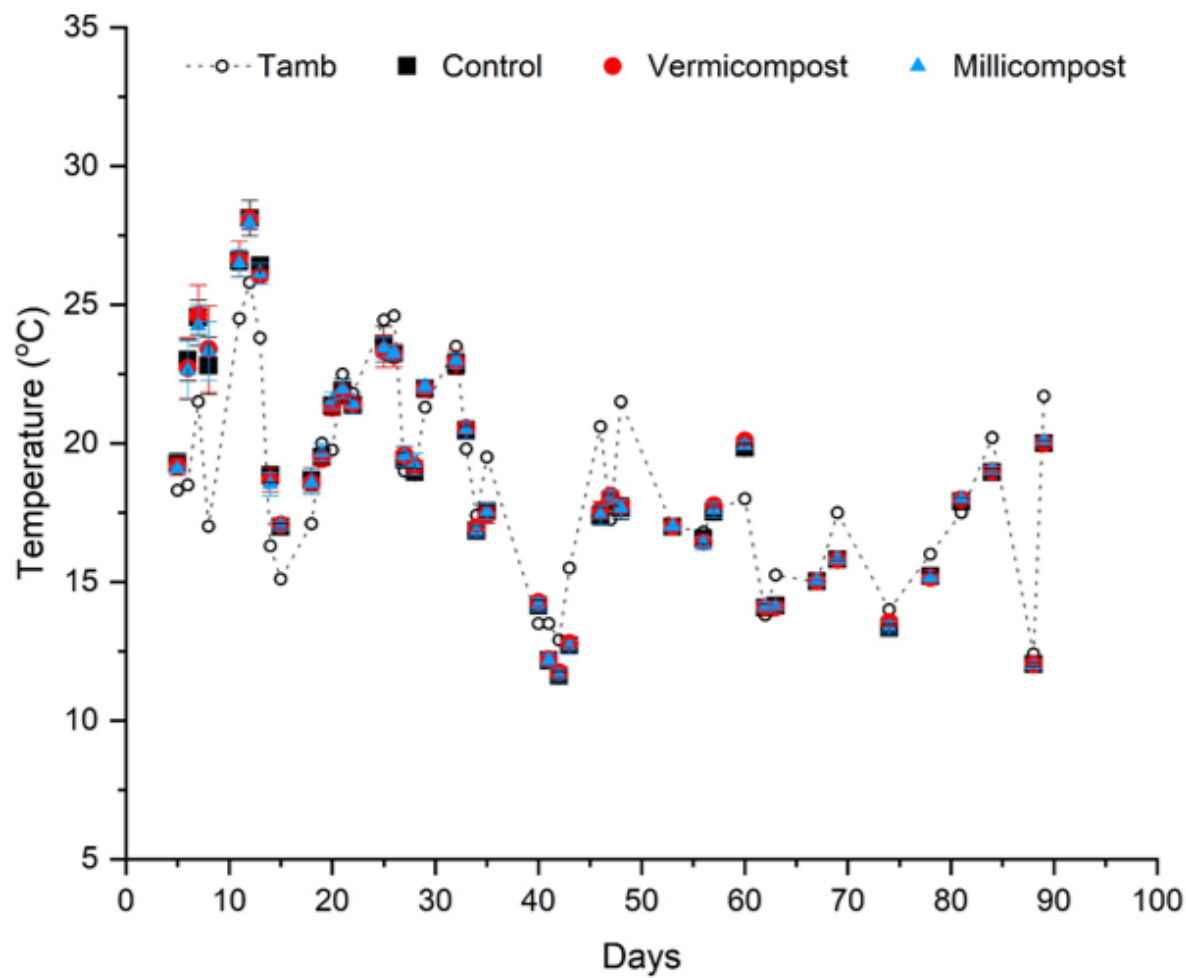


Figure 3

Temperature evolution for the three composting types (means and standard deviations; n=6). The dashed line marks the ambient temperature

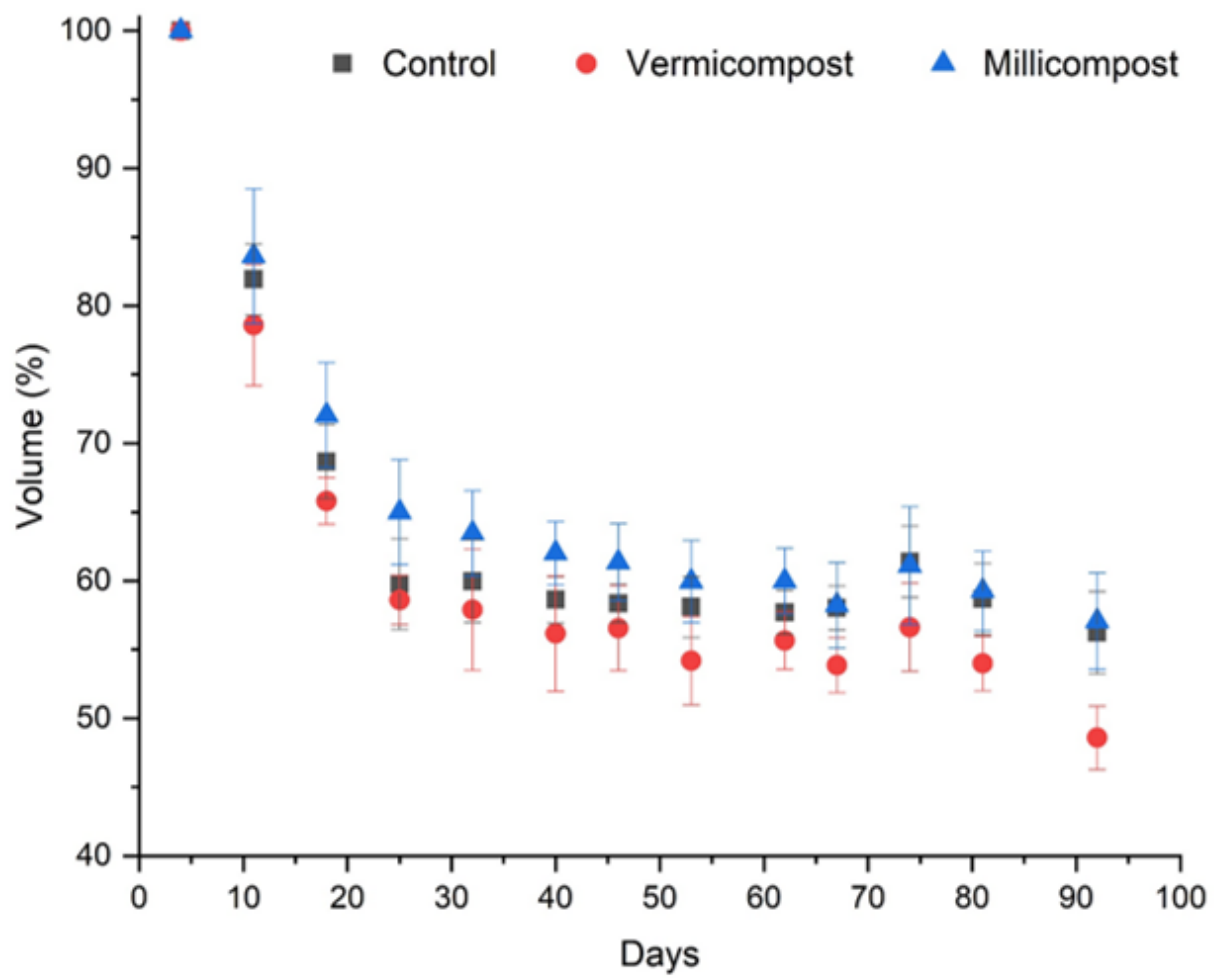


Figure 4

Volumes of the three composting types during 92 days (means and standard deviations; n=6)

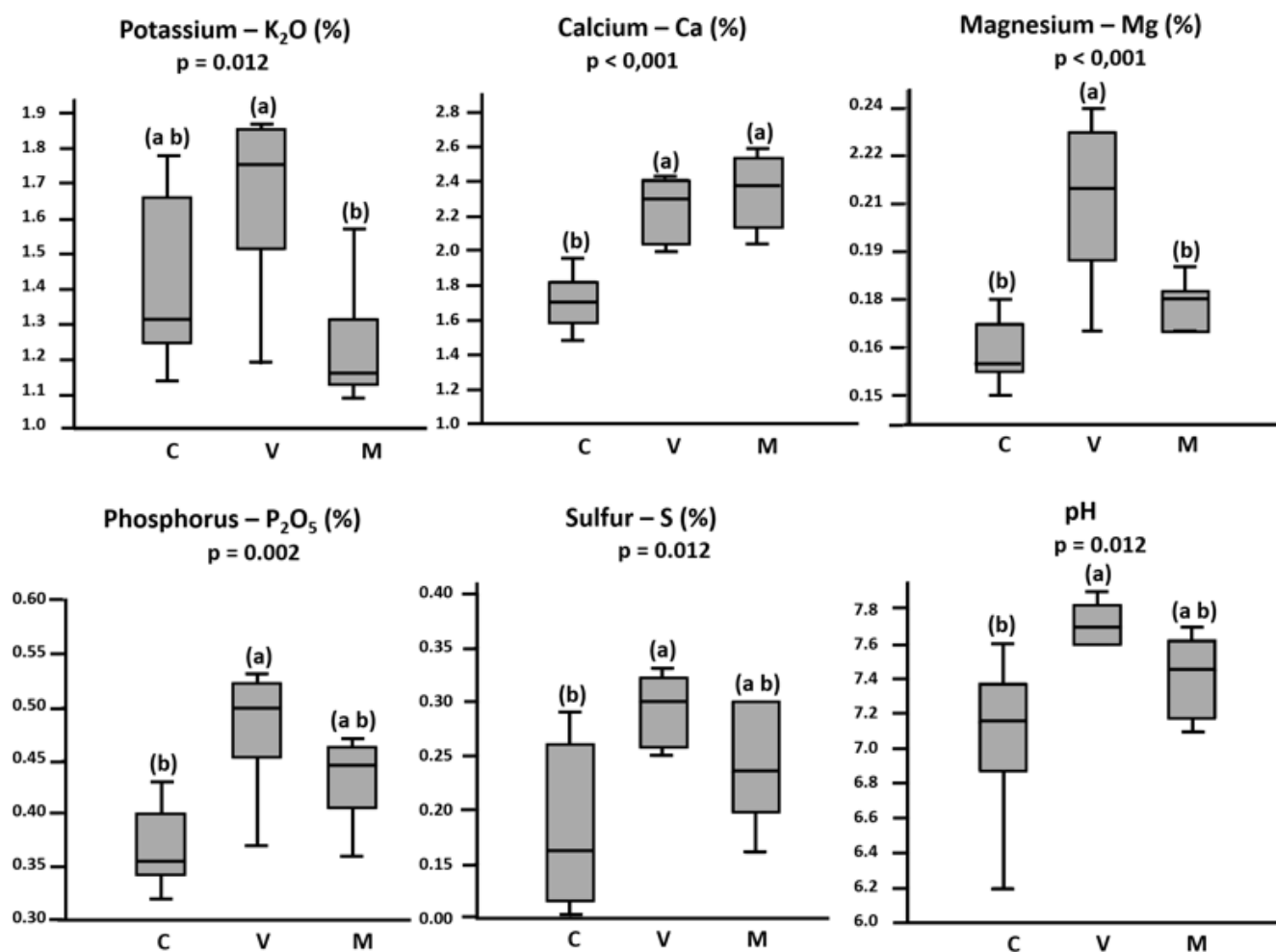


Figure 5

Chemical parameters obtained for the solid organic compounds (humus) resulting from the three composting types (V-vermicomposting, M-millicomposting and C-control). Boxplots show median and quartile values (n=6). ANOVA p-values indicated for each variable. Different letters indicate post-hoc significant differences (p < 0.05) according to the Tukey test (Dunn test for pH)

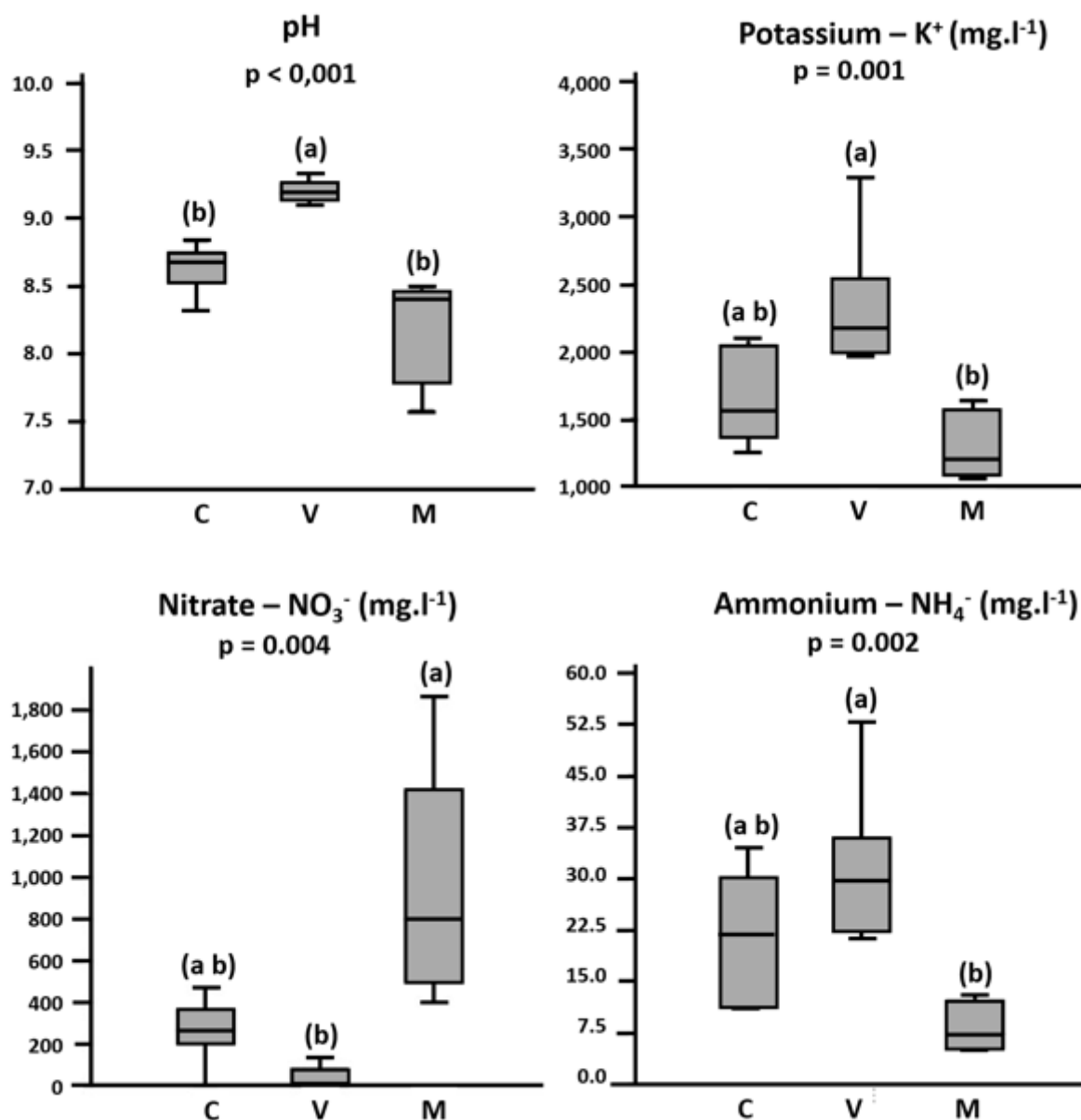


Figure 6

Physicochemical parameters of leachates resulting from the three composting types (V-vermicomposting, M-millicomposting and C-control). Boxplots show median and quartile values (n=6). ANOVA p-values indicated for each variable. Different letters indicate post-hoc significant differences (p <0.05) according to the Tuckey test (corrected Dunn test for pH and NO₃-)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [graphicalabstract5X13articleWBV17set21.pdf](#)